Public Health Lab (PHL) Mycobacteriology Lab Guidelines

This section describes the various types of lab tests done by the Washington State Public Health Lab, Mycobacteriology Lab for TB diagnosis and monitoring.

AFB Smears:

Acid Fast Bacilli (AFB) smears are performed on sputum or other non-respiratory specimens to detect the presence of *Mycobacterium*. The term "acid-fast" refers to the type of a staining technique used by the laboratory to make it possible to visualize the bacilli under the microscope. Smears are performed by the Auramine-O fluorescence method. This is more sensitive than the Kinyon and Ziehl-Neilson methods.

Smears are usually prepared and read within 24 hours of a working day after the specimen is received. Laboratory personnel then report the results to the submitter, the Local Health Jurisdiction (LHJ), and the State TB Program.

If the smear is positive for AFB, the health care provider may make a presumptive diagnosis of TB pending the results of the culture; however, persons with active TB may have negative AFB smears.

There are several other species of mycobacteria that are "acid-fast". Therefore, cultures must also be grown and tested to confirm the presence of ***M. tuberculosis complex.***

TB Cultures:

It is important to examine the culture on all specimens, whether or not the AFB smear is positive, as some clients with active TB disease may have negative AFB smears.

Culture is performed using solid medium and the BACTEC system. The BACTEC system uses a liquid media with a substrate containing radioactive tagged carbon. When bacteria grow in the media, the carbon in the media is metabolized and partially converted to radioactive CO_2. The vials are read weekly in a BACTEC 460TB instrument. This instrument detects the level of radioactive CO_2, and when the level of radiation reaches a pre-determined level, the vial is stained for AFB.

If the vial is positive for AFB, the organism is identified using genetic probes. Using the BACTEC and genetic probes can yield results in 10-14 days.

If the vial is negative for AFB, the specimen is held for 8 weeks allowing for growth to develop, before a negative result is reported.

Using solid medium and conventional identification methods, results can be available in 6-12 weeks.

A positive culture for *M. tuberculosis* confirms a diagnosis of TB, but health care providers can also diagnose TB based on signs, symptoms, radiography and response to therapy, if the culture is negative. A positive respiratory smear may indicate that the client is infectious. In addition, health care providers can also confirm diagnosis and progress by monitoring the client's improvement on treatment, and changes in the chest radiography.

Cultures should be repeated monthly until two consecutive specimens are culture negative.

Identifying Organisms:

Several identification methods can be used to identify the species of *Mycobacterium*:

GenProbe *Mycobacterium Tuberculosis* Direct test (MTD): this is a genetic amplification procedure used to identify *M. tuberculosis* complex directly from respiratory specimens. It has the advantage of providing a presumptive positive for TB without waiting for growth. If the specimen is AFB positive from a first time patient, the MTD test is performed. An MTD positive test result gives a high probability that the patient has *M. tuberculosis*. This is not a final result and must be confirmed by culture. This test can give results 24-48 hours after the smear is performed. Contact the lab for times the test is run

GenProbe Accuprobe: a genetic probe used for the rapid identification of *M. tuberculosis* complex, *M. avium* complex, *M. Gordonae*, and *M. kansasii*. When a BACTEC vial shows growth with a positive AFB smear, the Accuprobe test is performed to identify the organism. At the State Mycobacteriology Laboratory, all AFB-positive specimens are tested for *M. tuberculosis* and *M. avium* complexes. If the specimen shows yellow pigmentation, probes for *M. gordonae* and *M. kansasii* are done. GenProbe procedures are performed weekly and the results are called within 24 hours.

If the Accuprobe is negative, the specimen is inoculated on solid media for biochemical work-up. This may take up to 4 months or longer, after growth is obtained.

Drug Susceptibility Testing:

Drug susceptibility testing is performed on all first time positive patient specimens and on all positively identified *M. tuberculosis* cultures sent to the Public Health Laboratory (PHL).

Drug resistance should be identified as soon as possible to guide treatment. Susceptibility tests are repeated every 3 months for clients whose cultures remain positive, or as requested by the health care provider.

The State Mycobacteriology Lab uses two different methods for susceptibility testing.

- The BACTEC method (rapid method)
- The Disk Diffusion Agar Proportion method (plate method)

The BACTEC method (First Line Drugs):

The BACTEC method is used to determine resistance or susceptibility to five drugs:

- Isoniazid (INH)
- Rifampin (RIF)
- Streptomycin (SM)
- Ethambutol (EMB)
- Pyrazinamide (PZA)

The first 4 drugs are handled differently than the PZA. Five vials are inoculated with a suspension of the organism. Four of the vials each contain one of the first four drugs. The remaining vial, which does not contain a drug, is used as a control. Results are usually available within 1-2 weeks.

PZA uses different media with different growth requirements. PZA results are also available within 1-2 weeks.

The Disk Diffusion Agar proportion method (Second Line Drugs):

The Disk Diffusion method is used if an organism shows resistance to a drug tested by the BACTEC method. It confirms the results for drug resistance obtained from the BACTEC method and gives the percentage of resistance the organism has to a particular drug. Unlike the rapid method, Isoniazid, Streptomycin, and Ethambutol are tested for two concentrations on the plate method.

The following drugs are used for plate susceptibility test:

- Isoniazid (INH)
- Rifampin (RIF)
- Streptomycin (Strep)
- Ethambutol (EMB)
- Ethionamide (ETH or EA)
- P-aminosalicyclic acid (PAS)
- Ofloxacin (OFX)
- Amikacin (AN)

The Disk Diffusion method is performed on solid media. Each plate is divided into four quadrants, each containing a different drug at different concentrations, with one quadrant per plate with no drugs as a control. Dilutions of the organism are placed on each quadrant. If there

is growth on the quadrants containing the drug, the colonies are counted and resistance is reported as a percentage of growth on the drug quadrant vs. the growth on the control quadrant. Results are usually available within 3-4 weeks.

Using Other Labs:

For test procedures not done at the State PHL Mycobacteriology Lab (i.e. drug susceptibility test for non-TB isolates or for extended second line drug susceptibility for TB isolates), grown cultures can be sent to CDC or National Jewish lab.

Requests to send specimens to the CDC must be submitted through the State PHL Mycobacteriology Lab, with the appropriate paperwork. Use the CDC specimen request form.

Note: The CDC does not test for drug susceptibilities on *M. avium*. National Jewish will perform the test for a fee.

Requests to send specimens to National Jewish may be facilitated through PHL. National Jewish charges a fee for services, and the submitter must fill out a request form and arrange for payment. Use the National Jewish Mycobacteriology Request Form. The State Mycobacteriology Lab will not fill out the form, but will send the specimen and completed form to National Jewish for the health care provider.

Specimen Collection Kits and Regulations

Kits for submitting clinical specimens to PHL are provided to submitters by the Washington State Public Health Laboratories mailroom.

Order tuberculosis specimen kits and lab slips from:

Washington State Public Health Laboratories
Mailroom
1610 NE 150th St
Shoreline, WA 98155

Phone: 206-361-2865
Fax: 206-361-4997

Print and use black ink to fill out the Mycobacteriology laboratory form completely with:

- Date specimen was obtained
- Type of test requested
- Gender
- Patient date of birth
- Patient name – last, first, middle

- Patient mailing address
- Submitter name
- Submitter mailing address
- Submitter phone number
- Submitter Identification number for patient, if applicable
- Results and dates of any testing done at submitter lab, if applicable

Wrap the labeled specimen tube in the absorbent material, put in the plastic bag and insert into the aluminum canister. Package and ship the specimen according to current shipping regulations. See the Department of Transportation Specimen Shipping Regulations.